Long-Term Efficacy of Leptin Replacement in Patients With Generalized Lipodystrophy

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Ectopic fat accumulation has been implicated as a contributing factor in the abnormal metabolic state of obesity. One human model of ectopic fat deposition is generalized lipodystrophy. Generalized lipodystrophy is a rare disorder characterized by a profound deficiency of adipose tissue with resultant loss of triglyceride storage capacity and reduced adipokines, including leptin. Subjects with generalized lipodystrophy and reduced leptin levels often have an increased appetite leading to hyperphagia. Excess fuel consumption, coupled with a lack of adipose tissue, contributes to the significant ectopic triglyceride accumulation in the muscle and liver seen in these subjects. This ectopic fat, along with the deficiency in leptin signaling and perhaps other adipokines, likely contributes to insulin resistance, diabetes, and hepatic steatosis. We report here the long-term effects of leptin replacement in a cohort of these subjects. Fifteen patients with generalized lipodystrophy were treated with twice-daily recombinant methionyl human leptin (r-metHuLeptin) for 12 months. We evaluated metabolic parameters at baseline and every 4 months. Antidiabetes medications were decreased or discontinued as necessary. Reductions were seen in serum fasting glucose (from 205 ± 19 to 126 ± 11 mg/dl; $P < 0.001$), HbA_{1c} (from 9 ± 0.4 to 7.1 ± 0.5%; $P < 0.001$), triglycerides (from 1,380 ± 500 to 516 ± 236 mg/dl; $P < 0.001$), LDL (from 139 ± 16 to 85 ± 7 mg/dl; $P < 0.01$), and total cholesterol (from 284 ± 40 to 167 ± 21 mg/dl; $P < 0.01$). HDL was unchanged (from 31 ± 3 to 29 ± 2 mg/dl; $P = 0.9$). Liver volumes were significantly reduced (from 3,663 ± 326 to 2,190 ± 159 cm^3; $P < 0.001$), representing loss of steatosis. Decreases were seen in total body weight (from 61.8 ± 3.6 to 57.4 ± 3.4 kg; $P = 0.02$) and resting energy expenditure (from 1,929 ± 86 to 1,611 ± 101 kcal/24 h; $P < 0.001$). R-metHuLeptin led to significant and sustained improvements in glycemia, dyslipidemia, and hepatic steatosis. Leptin represents the first novel, effective, long-term treatment for severe forms of lipodystrophy.
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AGL, acquired generalized lipodystrophy; CGL, congenital generalized lipodystrophy; NAFLD, nonalcoholic fatty liver disease; PPAR, peroxisome proliferator–activated receptor; REE resting energy expenditure; r-metHuLeptin, recombinant methionyl human leptin.

The lipodystrophies represent a group of clinical syndromes characterized by various degrees of adipocyte loss. In the generalized forms, adipocyte loss is the most severe. The discovery of leptin introduced a new concept in energy regulation (1). This formed the basis for understanding that adipose tissue is an endocrine organ and that generalized lipodystrophy in rodents and patients constituted a severe hypoleptinemic state (2,3). The metabolic phenotype is characterized by extreme dyslipidemia, insulin resistance, and diabetes (4).

The production of recombinant leptin provided the opportunity to determine whether leptin replacement therapy in a leptin-sensitive state would improve this extreme metabolic phenotype. First in rodent models (5) and then in preliminary short-term studies in humans (6), it was shown that recombinant leptin administration could ameliorate dyslipidemia, insulin resistance, and diabetes. Until now, however, there have been no substantial data demonstrating the long-term effectiveness of leptin replacement therapy. In the present study, we demonstrate that recombinant leptin has a potent and sustained effect to reverse the severe metabolic features of generalized lipodystrophy and that leptin represents the first novel treatment for extreme insulin resistance since insulin was introduced >80 years ago.

RESEARCH DESIGN AND METHODS

We evaluated 27 consecutive patients with generalized lipodystrophy secondary to either congenital generalized lipodystrophy (CGL) or acquired generalized lipodystrophy (AGL). Inclusion criteria for recombinant methionyl human leptin (r-metHuLeptin) therapy included hypoleptinemia (male patients <4 ng/ml, female patients <5 ng/ml), metabolic abnormalities such as hypertriglyceridemia (>200 mg/dl), hyperinsulinemia (>30 μU/ml), diabetes, and ability to adhere to the leptin therapy and protocol regime. A total of 20 patients met the inclusion criteria to initiate therapy with r-metHuLeptin. Of these patients, 15 have completed at least 1 year of treatment and are presented in this analysis. Five patients who were withdrawn from r-metHuLeptin therapy before 12 months are discussed later. The first six patients were presented in the initial 4-month study (6).

R-metHuLeptin therapy was given as a self-administered, twice-daily subcutaneous injection as previously described (6). The dose was escalated to the full dose over the first 2 months of treatment. Thereafter, the usual replacement dose was 0.06–0.08 $mg \cdot kg^{-1} \cdot day^{-1}$ for females and 0.04 $mg \cdot kg^{-1} \cdot day^{-1}$ for males in an attempt to simulate the normal to high physiologic range. Patients were evaluated at the Clinical Center of the National Institutes of Health at baseline and every 4 months for 1 year. Inpatient data were collected on a metabolic unit during each visit. At baseline, patients were on aggressive conventional treatment for diabetes and dyslipidemia. These medications were subsequently lowered or discontinued if indicated. Results were analyzed as a function of baseline. The protocol was approved by the institutional review board of the National Institute of Diabetes and Digestive

TABLE 1
Baseline clinical characteristics of patients

Patient no.*	Age (years)/sex (M/F)	Type of lipodystrophy	Serum leptin (ng/ml)†	Weight (kg)	BMI (kg/m^2)	Body fat (%)‡	REE (kcal/24 h)
NIH-1	17/F	AGL at age 12	1.5	34.4	14.6	5.7	2,010
NIH-2	17/F	CGL	2.5	68.6	24.0	6.0	2,030
NIH-3	27/F	AGL at age 3	1.7	64.5	20.2	10.1	1,570
NIH-4	17/F	CGL	1.2	63.5	21.4	7.9	2,480
NIH-5	16/F	CGL	0.8	76.2	25.4	7.3	2,350
NIH-6	36/F	CGL	<0.5	55.7	21.9	7.0	1,370
NIH-8	40/F	CGL	1.4	55.1	18.1	7.6	1,590
NIH-9	13/F	AGL at age 6	4.0	31.0	15.4	8.0	1,690
NIH-11	13/M	CGL	1.3	73.5	23.6	7.4	1,770
NIH-13	12/F	CGL	0.8	69.8	23.0	7.7	2,300
NIH-16	47/F	CGL	1.2	60.4	22.9	9.0	1,990
NIH-19	29/F	AGL at age 28	1.3	55.4	19.3	6.9	2,390
NIH-20	23/F	CGL	1.4	80.6	25.2	7.8	1,840
NIH-22	14/F	CGL	3.3	69.9	23.4	14.2	1,810
NIH-24	17/M	CGL	0.9	68.4	24.1	5.8	1,740
Mean ± SE	23 ± 3	—	1.6 ± 0.2	61.8 ± 3.6	21.5 ± 0.9	7.9 ± 0.5	1,929 ± 86

*NIH numbers correspond to previous publications. †Normal fasting range of leptin for male patients: 3.8 ± 1.8 ng/ml; female patients: 7.4 ± 3.7 ng/ml (37). ‡Previous reports on patients NIH-1 through NIH-6 show higher values for percent body fat due to a recording error in these two previous studies.

and Kidney Diseases. Informed consents were obtained from each patient or his/her legal guardian.

Biochemical analyses. Serum leptin levels were determined by immunoassays with the use of a commercial kit (Linco Research, St. Charles, MO). HbA_{1c} (A1C) values were measured by ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). Serum thyroid-stimulating hormone, growth hormone, IGF-1, and C-reactive protein levels were measured with a two-site chemiluminscent immunometric assay on DPC Immulite 2000 equipment (Diagnostic Products). Free thyroxine (FT_4) was measured with an electrochemiluminescent competitive immunoassay on Elecsys 2010 equipment (Roche Diagnostics, Indianapolis, IN). Insulin was determined by immunoassay (Abbott Imx Instrument, Abbott Park, IL). Serum glucose and lipid values were determined according to standard methods with the use of automated equipment (Beckman, Fullerton, CA). All values represent morning fasting levels.

Procedures. To assess changes in caloric and macronutrient intake, subjects completed the Block 1998 Revision of the Health Habits and History Questionnaire, a self-administered standardized semiquantitative food frequency questionnaire, at baseline and each subsequent visit to reflect intake between visits (7). Resting energy expenditure (REE) (Deltatrac equipment; Sensormedics, Yorba Linda, CA) was measured between 6:00 and 8:00 A.M. after an overnight fast of at least 8 h, whereas the patients remained at rest. After an overnight fast, each patient underwent an oral glucose tolerance test in which 1.75 g/kg up to 75 g dextrose was administered. A high-dose insulin tolerance test was performed with the use of 0.2 units of regular insulin per kilogram administered intravenously to assess the patients' sensitivity to insulin. The *K* constant (the rate of glucose disappearance as a reflection of the body's overall sensitivity to insulin) was calculated as the rate constant for the decrease in blood glucose levels after the intravenous administration of insulin with the use of first-order kinetics (8). Percent body fat was determined using dual-energy X-ray absorptiometry (QDR 4500; Hologic, Bedford, MA). Values for percent fat recorded in Oral and colleagues (6,9) for patients NIH-1 through NIH-6 are higher than those reported here due to a recording error in those two previous publications. Axial T1-weighted magnetic resonance imaging of the patients' livers was performed with the use of a 1.5-T scanner (General Electric Medical Systems, Milwaukee, WI). The liver volumes were estimated using the MEDx image analysis software package (Sensor Systems, Sterling, VA).

Statistical analyses. Values are expressed as means ± SE. SigmaStat (SPSS, Chicago, IL) was used to calculate a one-way ANOVA for repeated measures. If the data for any of the parameters were not normally distributed, a $\log_{10}$ transformation was done before analysis. Paired *t* tests were also used to compare results whenever applicable. A P value <0.05 was accepted as statistically significant.

RESULTS

Baseline characteristics. A total of 15 patients with severe forms of lipodystrophy were studied (Table 1). Eleven patients had CGL and 4 had AGL. The mean age of the patients was 23 years (range 12–47). Patients had markedly decreased body fat (mean 7.9% [range 5.7–14.2]) and were hypoleptinemic (1.6 ± 0.2 ng/ml). They consistently reported voracious appetites and had elevated REE (1,929 ± 86 kcal/24 h) (Table 1).

All 15 patients had uncontrolled diabetes (fasting glucose ≥126 mg/dl and/or A1C ≥ 7%), despite attempts to optimize therapy with oral hypoglycemics and/or insulin (Table 2). All but one patient had hypertriglyceridemia >150 mg/dl (mean 1,380 mg/dl [range 95–7,420 mg/dl]) despite therapy with fibrates. Patients had mild to extreme hepatomegaly (mean 3,663 ml [range 1,754–6,736]), consistent with nonalcoholic fatty liver disease (NAFLD). There were no consistent abnormalities in inflammatory markers such as C-reactive protein (Table 2).

Female patients generally had hypogonadotropic amenorrhea, as previously described (9,10). Additionally, both male and female patients had a suppressed growth hormone axis with low IGF-1. Despite these abnormalities, patients generally had normal growth and secondary sexual development. There were no consistent abnormalities in thyroid function tests (Table 2).

R-metHuLeptin therapy. Patients enrolled in an open-label pilot study of r-metHuLeptin for their severe insulin resistance, diabetes, dyslipidemia, fatty liver disease, and hypothalamic abnormalities. They were observed for 12 months. During this period, serum leptin levels increased and were sustained in a range to simulate normal (Table 3). Several patients developed binding antibodies, which act to increase the half-life of r-metHuLeptin and cause elevations in their serum levels. These binding antibodies show no evidence of neutralizing activity.

Subjects consistently reported a dramatic reduction in

TABLE 2
Baseline laboratory parameters of patients

Patient no.	Fasting glucose (mg/dl)	A1C (%)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Total cholesterol (mg/dl)	Growth hormone (ng/ml)	IGF-1 (ng/ml)	TSH (μIU/ml)	Free thyroxine (ng/dl)	C-reactive protein (mg/dl)	Liver volume (cc)
NIH-1	263	8.0	7,420	18	260	740	0.1	42	2.13	1.1	3.00	4,213
NIH-2	204	9.8	523	42	174	294	0.7	187	1.16	1.0	0.40	3,079
NIH-3	203	9.3	450	22	82	163	0.1	243	1.11	1.1	0.46	1,754
NIH-4	113	7.6	322	40	179	259	1.9	323	2.60	1.1	0.39	3,759
NIH-5	212	9.6	944	34	120	240	0.2	123	0.46	0.8	0.60	4,794
NIH-6	173	9.5	731	32	134	228	0.2	130	1.35	1.3	0.39	2,895
NIH-8	128	7.6	471	48	222	308	0.3	166	1.00	0.7	0.59	2,029
NIH-9	402	10.6	2,984	20	165	374	0.7	49	1.00	1.0	0.81	4,291
NIH-11	147	9.3	95	46	107	161	0.8	506	1.04	1.1	2.90	2,198
NIH-13	158	7.2	261	25	87	131	0.2	113	4.56	1.2	0.49	3,343
NIH-16	183	7.0	1,543	34	*	223	0.1	63	1.44	0.9	0.39	3,837
NIH-19	215	8.0	438	21	94	170	0.7	174	0.04	1.0	0.51	4,780
NIH-20	142	8.7	702	36	58	264	1.8	148	0.45	1.0	0.39	3,919
NIH-22	290	13.0	3,385	14	*	493	0.4	199	1.15	1.0	0.42	6,736
NIH-24	242	10.2	433	26	119	215	0.4	101	0.98	1.3	0.39	3,318
Mean ± SE	205 ± 19	9.0 ± 0.4	1,380 ± 500	31 ± 3	139 ± 16	284 ± 40	0.6 ± 0.1	171 ± 31	1.36 ± 0.28	1.0 ± 0.0	0.81 ± 0.23	3,663 ± 326

*Lipemic sample did not have direct LDL assay.

their appetite. This improvement in satiety was sustained throughout r-metHuLeptin therapy, leading to moderate reductions in weight (from 61.8 to 57.4 kg, $P = 0.02$), BMI (from 21.5 to 20 kg/m^2, $P < 0.001$), and REE (from 1,929 to 1,611 kcal/24 h, $P < 0.001$). These reductions were significant after 4 months, without further significant change from 4 to 12 months.

Most female patients resumed and sustained normal menses following initiation of r-metHuLeptin consistent with normalization of their gonadotropin axis (9,10). Other subjective changes, such as changes in energy or heat tolerance, were inconsistent. Furthermore, both thyroid and adrenal (data not shown) functions were normal at baseline and did not change with r-metHuLeptin therapy. Despite no significant change in fasting growth hormone levels, IGF-1 levels significantly increased, consistent with improvement in insulin sensitivity.

Effect on lipids. Patients had severe hypertriglyceridemia at baseline. R-metHuLeptin led to a 63% reduction in mean triglycerides (from 1,380 to 516 mg/dl, $P < 0.001$) (Fig. 1*A*). The most dramatic reductions were seen after 4 months and were more gradual thereafter. These reductions, however, did not normalize values in most patients. Additionally, LDL levels decreased significantly (from 139 to 85 mg/dl, $P = 0.01$), as did total cholesterol (from 284 to 167 mg/dl, $P < 0.001$). Interestingly, HDL levels were low at baseline and did not change over the course of 12 months (from 31 to 29 mg/dl, NS). Percent body fat decreased significantly (from 7.9 to 6.7%, $P = 0.002$). A large portion of fat loss was from the liver (volume change from 3,663 to 2,190 ml, $P < 0.001$) (11,12). Significant reductions were previously seen in intramyocellular fat content (11).

Effect on glycemic control. All patients had diabetes before r-metHuLeptin therapy. Leptin led to significant reductions in fasting glucose (from 205 to 126 mg/dl, $P < 0.001$) and A1C (from 9.0 to 7.1%, $P < 0.001$) (Fig. 1*B* and *C*). Again, the largest reductions were seen after 4 months and were more gradual thereafter. Insulin sensitivity significantly improved during treatment. The *K* constant rate of glucose disposal derived from insulin tolerance tests (Fig. 2*A* and *B*) more than doubled (from 0.0074 to 0.015, $P < 0.001$). This was despite the fact that two patients did not have insulin tolerance testing at 8 or 12 months because of hypoglycemia (<40 mg/dl) during 4-month testing.

Improvement in insulin sensitivity was further reflected in diabetic management during the course of r-metHuLeptin therapy. Before r-metHuLeptin therapy, 9 of 15 patients were on insulin with a mean dose of 916 units daily (Table 4). During r-metHuLeptin therapy, six patients were able to discontinue insulin due to euglycemia. The three patients still requiring insulin were able to decrease their mean dose by 65%. Likewise, six of eight patients were able to discontinue oral agents (primarily metformin) during r-metHuLeptin therapy.

Glucose tolerance tests were markedly abnormal at baseline (Fig. 3). After 4 months of r-metHuLeptin therapy, both fasting and 2-h levels were significantly reduced, approaching nondiabetic levels. These results were sustained throughout 12 months of r-metHuLeptin therapy. We also examined a subset of six patients who were not treated with insulin. Their baseline and 12-month glucose curves were similar to the entire cohort (Fig. 4*A*). Addi-

TABLE 3
Physical and biochemical changes during leptin

Test	Baseline	4 months	8 months	12 months	*P* value
Leptin (ng/ml)	1.6 ± 0.2	17.0 ± 3.8*	23.9 ± 6.8†	21.1 ± 4.8†	<0.001
Fasting glucose (70–99 mg/dl)	205 ± 19	163 ± 30	157 ± 30‡	126 ± 11†	<0.001
A1C (4.8–6.4%)	9.0 ± 0.4	7.6 ± 0.6*	7.0 ± 0.4†	7.1 ± 0.5†	<0.001
Triglycerides (<150 mg/dl)	1,380 ± 500	708 ± 286†	482 ± 233†	516 ± 236†	<0.001
HDL cholesterol (mg/dl)	31 ± 3	31 ± 2	29 ± 2	29 ± 2	0.9
LDL cholesterol (65–99 mg/dl)	139 ± 16	111 ± 15	97 ± 11‡	85 ± 7*	0.01
Total cholesterol (100–200 mg/dl)	284 ± 40	206 ± 37	164 ± 20*	167 ± 21*	<0.001
Fasting growth hormone (0.0–10.0 ng/ml)	0.6 ± 0.1	1.6 ± 0.5‡	2.1 ± 1.1	1.8 ± 0.5‡	0.3
IGF-1 (182–780 ng/ml)	171 ± 31	268 ± 46*	263 ± 41‡	274 ± 44‡	0.005
C-reactive protein (<0.80 mg/dl)	0.81 ± 0.23	0.69 ± 0.19	0.85 ± 0.32	0.67 ± 12	0.4
TSH (0.4–4.0 μIU/ml)	1.36 ± 0.28	1.15 ± 0.27	1.27 ± 0.30	1.25 ± 0.36	0.5
Free thyroxine (0.8–1.9 ng/dl)	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	0.8
Liver volume (ml)	3,663 ± 326	2,834 ± 276†	2,453 ± 242†	2,190 ± 159†§	<0.001
Weight (kg)	61.8 ± 3.6	58.9 ± 3.6*	57.8 ± 3.4*	57.4 ± 3.4*	0.02
BMI (kg/m^2)	21.5 ± 0.9	20.7 ± 0.9‡	20.2 ± 0.8*	20.0 ± 0.8*	<0.001
Body fat (%)	7.9 ± 0.5	7.7 ± 0.5	7.6 ± 0.5‡	6.7 ± 0.3*§	0.002
Energy expenditure (kcal/24 h)	1,929 ± 86	1,684 ± 81‡	1,581 ± 83†	1,611 ± 101‡	<0.001

*$P < 0.01$, †$P < 0.001$, ‡$P < 0.05$ compared with baseline; §$P < 0.05$ compared with 4 months. TSH, thyroid-stimulating hormone.

tionally, we examined their insulin responses to the glucose loads (Fig. 4*B*). Compared with baseline, insulin levels peaked earlier and the overall amount of insulin secreted in response to glucose at 12 months was less, consistent with improvement in insulin responsiveness and sensitivity.

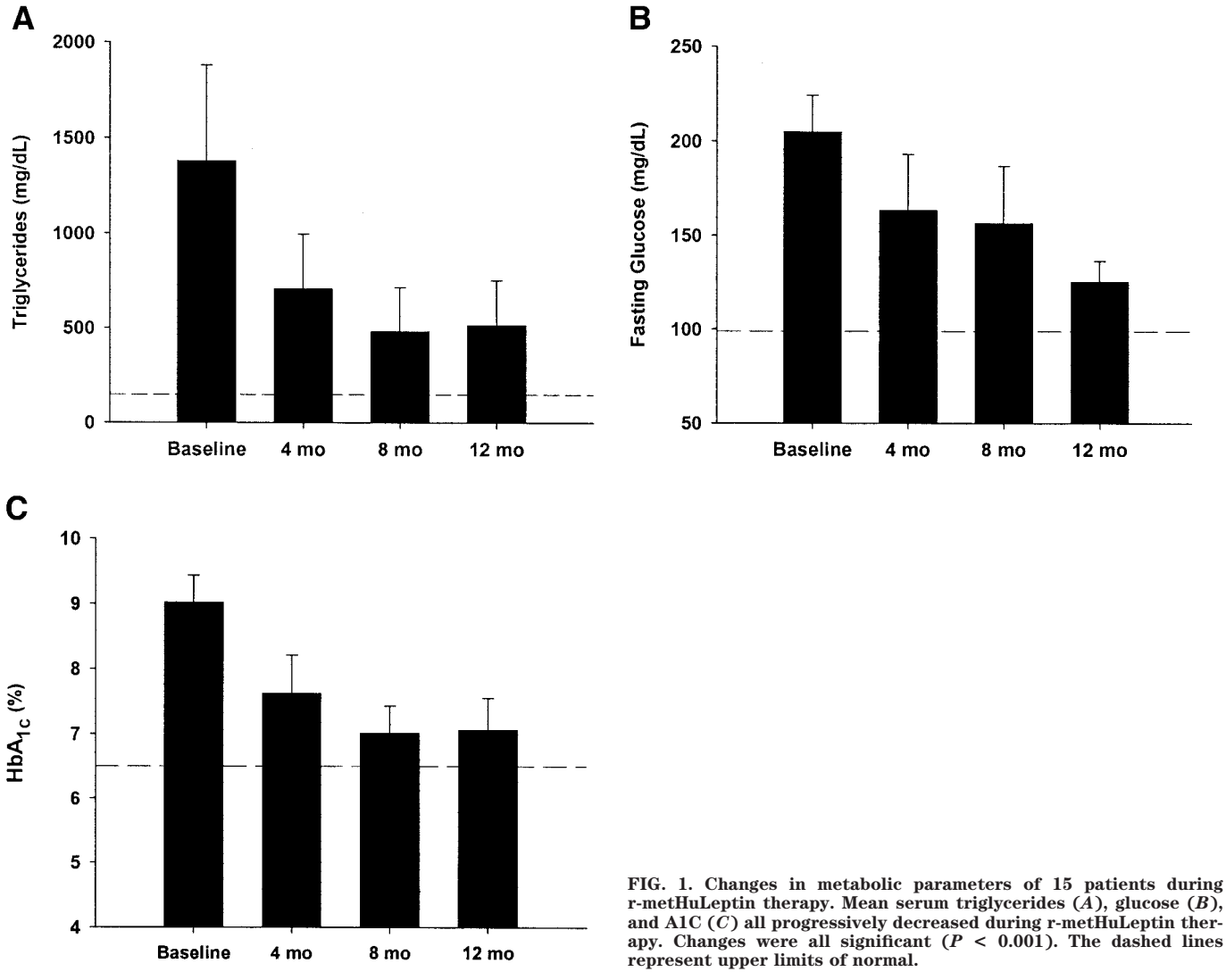

FIG. 1. Changes in metabolic parameters of 15 patients during r-metHuLeptin therapy. Mean serum triglycerides (*A*), glucose (*B*), and A1C (*C*) all progressively decreased during r-metHuLeptin therapy. Changes were all significant ($P < 0.001$). The dashed lines represent upper limits of normal.

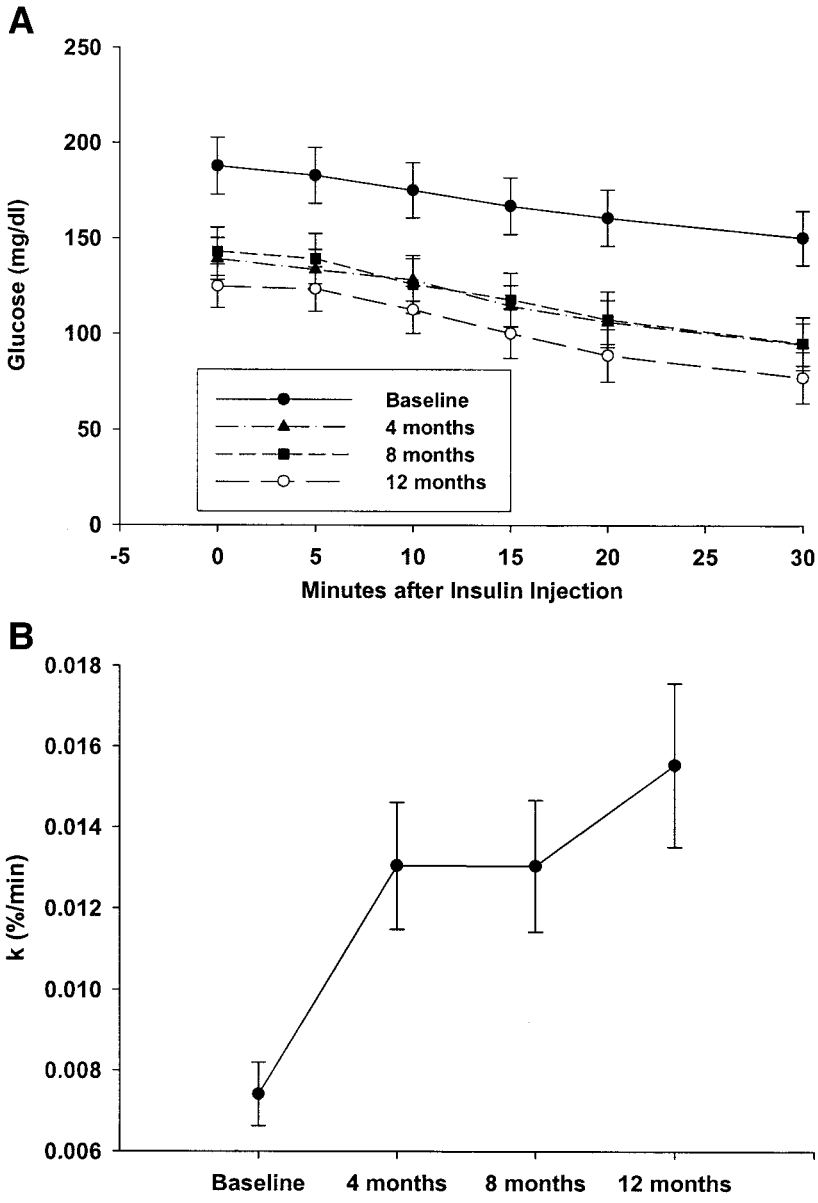

FIG. 2. Changes in insulin sensitivity in 15 patients during r-metHuLeptin therapy. *A*: Serum glucose levels were measured before and after 0.2 units of insulin per kg at baseline and during r-metHuLeptin therapy. The difference between baseline and subsequent measurements was significant (<0.001). The rate of glucose disposal, represented by the *K* constant (*B*), was increased significantly ($P < 0.001$) during r-metHuLeptin therapy. The values at 8 and 12 months represent 13 patients, since 2 patients experienced severe hypoglycemia (<40 mg/dl) at 4 months.

Patients withdrawn from protocol before 12 months. R-metHuLeptin was well tolerated by all subjects. R-metHuLeptin was discontinued before 12 months in the following five subjects who developed an event that was either not readily explainable or likely to preclude successful completion of the study.

NIH-10 is an 8-year-old female with AGL who discontinued leptin after 1 month following an acute rise in liver enzymes, later believed to be secondary to concurrent amoxicillin/clavulanate use. Leptin was resumed and then discontinued 3.5 months later following an episode of acute respiratory distress. These episodes recurred after leptin was stopped, and she was diagnosed with vocal cord dysfunction and asthma. Leptin was later resumed, and she is currently tolerating it well.

TABLE 4
Antidiabetic therapy of patients during leptin therapy

	Baseline	12 months
Patients on insulin	9/15	3/15
Mean daily insulin dose (units)	916	322
Range of daily insulin dose (units)	50–5,000	40–750
Patients on oral agents	8/15	2/15

NIH-14 is a 35-year-old male with AGL who stopped leptin at 8 months due to worsening proteinuria and diagnosis of membranoproliferative glomerulonephritis. This case was previously reviewed (13). His 8-month evaluation demonstrated improvement in his metabolic parameters consistent with the group data. Now on hemodialysis, he has resumed r-metHuLeptin therapy with improvement in metabolic parameters.

NIH-15 is a 68-year-old male with AGL who had diffuse lymphadenopathy diagnosed as "reactive lymphoid nodules" before r-metHuLeptin therapy. During r-metHuLeptin therapy, he had progression of lymphadenopathy. Repeat biopsy revealed T-cell lymphoma, and leptin was discontinued at 8 months. His 8-month evaluation also demonstrated improvement in his metabolic parameters consistent with the group data.

NIH-25 was a 15-year-old female with CGL. After 3

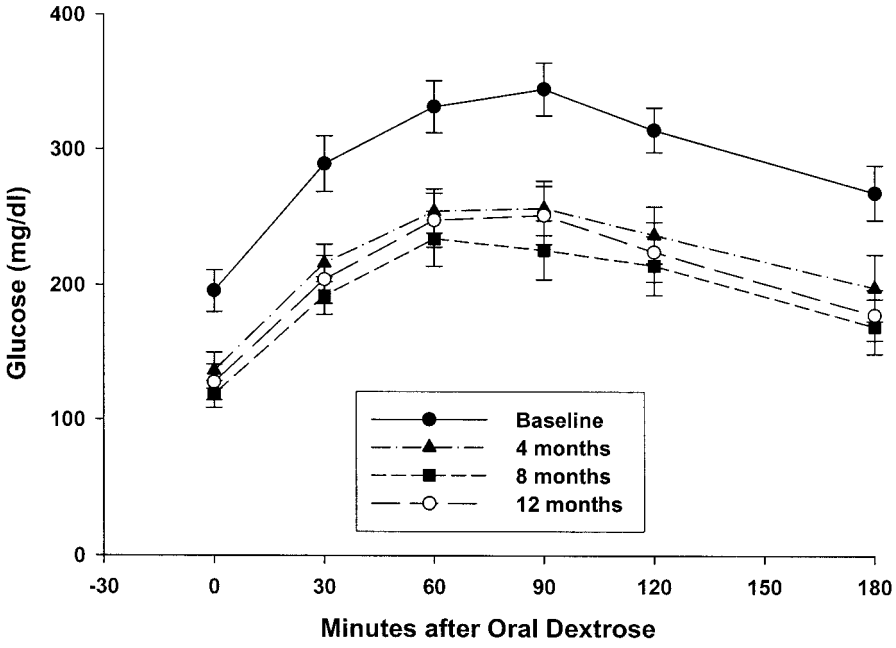

FIG. 3. Change in oral glucose tolerance in 14 patients during r-metHuLeptin therapy. Serum glucose levels were measured before and after 1.75 g/kg up to 75 g dextrose at baseline and during r-metHuLeptin therapy. One patient with type 1 diabetes was not tested. The difference between baseline and subsequent measurements was significant (<0.001).

months of r-metHuLeptin therapy, she became noncompliant with all her medications, including leptin. She developed fulminant pancreatitis with sepsis and hypovolemic shock and expired despite aggressive treatment.

NIH-26 is a 51-year-old male with AGL. His course of r-metHuLeptin therapy was interrupted by multiple surgeries for peripheral vascular disease with prolonged hospitalization. He was evaluated only once at 8 months without demonstrable change from baseline and was subsequently withdrawn from the protocol.

DISCUSSION

In the present study, we have shown that r-metHuLeptin sustained its effectiveness in ameliorating hyperlipidemia, insulin resistance, and hepatomegaly throughout 12 months of therapy in 15 patients with generalized lipodystrophy. Nine of 13 patients initially using conventional treatments were able to discontinue their antidiabetes medications. The other four patients with more severe abnormalities were able to significantly reduce their medication requirements. Glucose tolerance tests demonstrated lower fasting insulin levels, a more rapid insulin peak, and a reduced amount of insulin required to maintain euglycemia, further supporting a decrease in insulin resistance.

We have used clinical criteria of both glucose tolerance tests and insulin tolerance tests to measure changes in insulin and glucose responsiveness as a function of r-metHuLeptin therapy. These parameters have been validated previously by the hyperinsulinemic-euglycemic clamp technique, showing that r-metHuLeptin increases insulin-stimulated peripheral glucose uptake and increases insulin suppression of hepatic glucose output (11).

The persistent effect of leptin on liver volume and percent body fat reached further statistical significance from 4 to 12 months on r-metHuLeptin. The reduction of hepatomegaly was associated with improvement of nonalcoholic steatohepatitis, a severe inflammatory manifestation of NAFLD (12). These data suggest that leptin had a progressive effect to diminish ectopic fat, which corresponded temporally with improvements in NAFLD and insulin resistance. These persistent effects have been seen in a subset of these patients followed for up to 4 years (data not shown).

The severe dyslipidemia in these patients was significantly improved. The greatest abnormality and improvement was in triglycerides, which were reduced by ~63%. Both total cholesterol and LDL cholesterol were moderately elevated at baseline, and both were significantly reduced with r-metHuLeptin. HDL cholesterol levels were uniformly low in these patients, correlating with the very high triglyceride levels. Interestingly, the reduction in triglycerides did not lead to an increase in HDL, even in patients whose triglycerides were normalized.

Although this is the largest cohort of patients with generalized lipodystrophy treated long-term with r-met HuLeptin, the power of the study is limited by its size. Other limitations include the nonrandomized open-label nature of the study and the combination of r-metHuLeptin use with conventional medications. Despite these limitations, our observations have been supported by a recent report from Japan (14).

Both genetic and acquired forms of generalized lipodystrophy represent a complex set of disorders. We have pointed out that five patients did not complete the 12-month study. A notable observation during this protocol was the prevalence of proteinuria and unique nephropathies of these patients (13). The progression of proteinuria during the study required the discontinuation of leptin before 12 months in one patient (NIH-14) and later in another two patients (NIH-2 and NIH-9). Although most patients' proteinuria improved during the course of r-metHuLeptin therapy, we cannot exclude the possibility that leptin therapy was associated with exacerbation of underlying renal disease in two patients with AGL (NIH-9 and NIH-14).

Generalized lipodystrophy leads to severe metabolic abnormalities, including hypertriglyceridemia, NAFLD, in-

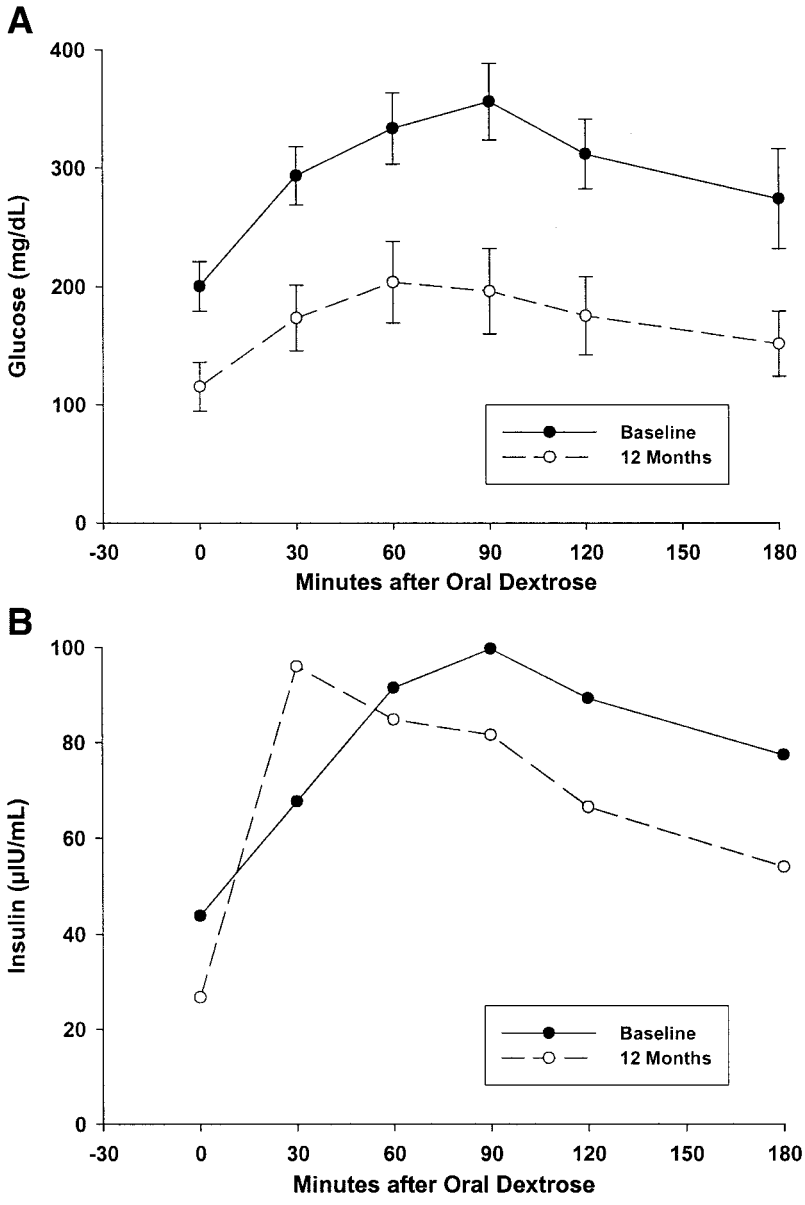

FIG. 4. Change in oral glucose tolerance in six patients not on exogenous insulin during r-metHuLeptin therapy. In this subset of patients not receiving exogenous insulin, serum glucose and insulin levels were measured before and after 1.75 g/kg up to 75 g dextrose at baseline and after 12 months of r-metHuLeptin therapy. *A*: The difference in serum glucose levels between groups was significant ($P < 0.01$). *B*: The mean serum insulin response to dextrose at 12 months peaked and declined earlier than at baseline with a lower area under the curve; however, this was not statistically significant.

sulin resistance, and diabetes. The lack of subcutaneous and visceral adipose tissue results in the storage of triglycerides in ectopic locations such as muscle and liver. The degree of ectopic fat accumulation is further compounded by hyperphagia, which is characteristic of the hypoleptinemic state in rodents and humans. Ectopic triglycerides usually signify the presence of long-chain free fatty acids, which are cytotoxic (15,16) and believed to interfere with normal insulin signaling leading to insulin resistance. This mechanism of insulin resistance is similar, albeit more severe, to that seen in obesity.

The phenotypes of three different clinical states that have been treated with leptin provide some insight into the mechanisms of leptin action. The first example is obesity (17), where the phenotype is excess fat stored in normal adipocytes with normal endocrine function. Often, there is moderate storage of ectopic fat and moderate insulin resistance. These patients are hyperleptinemic and leptin resistant, and leptin has a limited therapeutic effect.

The second example is congenital leptin deficiency (18–20), which is characterized by obesity, excessive adipose tissue, moderate storage of ectopic fat, and moderate insulin resistance. Here, leptin replacement therapy is highly effective in controlling appetite, reducing food intake, and reducing overall fat mass. Metabolic abnormalities of dyslipidemia, glucose intolerance, and steatosis, which are only mildly abnormal, are also corrected by leptin therapy. Obesity in this case is directly related to leptin deficiency, and the effect here is largely mediated by leptin's role in reducing energy intake.

The third example is generalized lipodystrophy, characterized by absence of adipose tissue with hypoleptinemia, hyperphagia, and excessive ectopic fat accumulation. These patients' hypertriglyceridemia and insulin resistance are more severe than what is seen in obesity or congenital leptin deficiency. R-MetHuLeptin therapy is associated with decreased energy intake, improvements in dyslipidemia, hepatic steatosis, and insulin resistance, as well as hypothalamic regulation of the gonadal axis (9,10).

One action of r-metHuLeptin is to mitigate the hyperpha-

gia in these patients. Leptin is known to act at the hypothalamus, inhibiting the synthesis and release of orexigenic peptides, including neuropeptide Y and agouti-related protein (21). When patients with lipodystrophy and reduced leptin were placed on r-metHuLeptin, they reported improved satiety and satiation, resulting in ~40% reduction of caloric intake (22). This decrease in energy consumption would potentially allow for mobilization of stored triglycerides.

A natural question is whether leptin's ability to decrease food intake adequately explains the metabolic improvements seen. Pair-feeding experiments in animal (5,23–25) and human (6) models of hypoleptinemia have attempted to answer this question. Although pair feeding may lead to some improvement in the metabolic profile compared with the ad lib state, it does not correct the metabolic abnormalities to the same extent as leptin replacement. This would suggest that the action of r-metHuLeptin is not mediated solely by reducing food intake. Based on the clearance of triglycerides from the muscle and the liver, one might speculate that one mechanism contributing to the beneficial effects is the removal of ectopic fat from the tissues. This would be consistent with the lipotoxicity hypothesis of Unger (26).

Recent insights suggest how leptin might further mediate loss of ectopic fat. In obesity and other insulin-resistant states, a predominant mechanism for ectopic fat accumulation appears to be mitochondrial dysfunction (27), leading to decreased fatty acid oxidation in tissues such as muscle and liver. Thus, triglycerides begin to accrue ectopically at a faster rate than they are utilized. Leptin is believed to be crucial in mitochondrial function. Both leptin deficiency and leptin resistance represent states of decreased leptin signaling, potentially leading to mitochondrial dysfunction. In rodent models, leptin increased expression of AMP-activated protein kinase (28) and peroxisome proliferator–activated receptor (PPAR) α (29), activating enzymes of fatty acid oxidation, including carnitine palmitoyl transferase-1 and acyl-CoA oxidase, and inhibiting enzymes of lipogenesis, including acyl-CoA carboxylase and stearoyl-CoA desaturase-1 (28,30). Additionally, leptin upregulates mitochondria biogenesis through expression of PPARγ coactivator-1α (29,31). Zucker diabetic fatty rats transfected with leptin had a dramatic augmentation in PPARγ coactivator-1α expression in white adipose tissue with resultant delipidation (31). Their shrunken adipocytes were filled with mitochondria, demonstrating conversion from fat-storing into fat-burning cells. Thus, leptin appears to enhance mitochondrial production and function, which likely accelerates the removal of ectopic fat.

R-metHuLeptin led to a reduction in appetite, weight, and REE. Patient weight reduction has been shown to involve reductions of both fat mass and lean body mass with no change in bone mass (32). These changes were apparent after 4 months of treatment, plateaued, and then maintained from the 4th to the 12th month of therapy.

Hyperinsulinemia leads to increased androgen production from the ovaries, forming the basis of polycystic ovarian syndrome. The women in the group had an extreme form of polycystic ovarian syndrome due to their extreme insulin resistance. R-metHuLeptin therapy led to a decrease in serum androgen levels and normal cyclic menses in women of reproductive age (9,10).

Leptin is the first and only adipokine to be administered to humans for long-term therapeutic purposes. Adiponectin is another adipokine that is associated with increased insulin sensitivity and fatty acid oxidation (33). Adiponectin levels, which are low in all forms of insulin resistance, were low to undetectable in our patients at baseline and unchanged following r-metHuLeptin therapy (data not shown). However, levels in the *ob/ob* mouse (34) and patients with congenital leptin deficiency (19) were increased following leptin replacement. The possibility that successful leptin signaling leads to adiponectin release is intriguing, but it is clear that adiponectin did not mediate the insulin sensitivity effect of leptin in our patients. Likewise, in one animal model, leptin alone produced a near-maximal effect (35), whereas a second model demonstrated a synergistic effect with adiponectin (33).

We conclude that r-metHuLeptin therapy has a sustained effect to improve insulin resistance, dyslipidemia, and hepatic steatosis in a hypoleptinemic group of lipodystrophic patients. This is in contrast to the hyperleptinemic state of obesity, in which leptin has a limited effect. Whether leptin would have an effect in a subset of obese patients with relatively lower leptin remains to be determined. We are attempting to answer this question in patients with partial forms of lipodystrophy and have presented preliminary data in patients with other forms of extreme insulin resistance (36). Though the potential larger role of recombinant leptin therapy remains to be determined, the present study represents the first novel, effective, long-term therapy for patients with a syndromic form of extreme insulin resistance.

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